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Extraction and Identification of Glycerides in Sesamum indicum Oil Seeds from Manipur

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Abstract

Seed oil from Sesamum indicum was obtained by solvent extraction technique where petroleum ether was used as the extracting solvent after crushing the kernels. The oil was purified before going to further analysis achieved through column chromatography using silica gel (60-120 mesh) and an eluent mixture of petroleum ether and ethyl acetate (20:1). Subsequently, transesterification is done on the purified oil to produce biodiesel, also known as fatty acid methyl ester (FAME). The FAME composition of Sesamum indicum oil was determined using NMR, IR, GCMS analysis. The FAME profile of Sesamum indicum oil consists of 15.35 wt. % methyl palmitate [C16:0], 67.03 wt. % of methyl linoleate [C18:2], 14.92 wt. % of methyl stearate [C18:0] and 2.70 wt. % of methyl arachidate [C20:0].

Keywords: *Thoidong, Sesamum Indicum, Transesterification, Biodiesel*

1. Introduction

Manipur boasts a rich variety of plant species, some of which produce seeds with high non-edible oil content. These plants thrive in both the plains and hilly regions of Manipur, India. But most of the plants are undervalued due to their lack of economic significance. Consequently, oil derived from such plants have limited commercial applications, leading to the gradual disappearance of these plants, as they are deemed unimportant by farmers, public and government entities [1].

This decline in plant diversity is exacerbated by concerns that large scale production of biodiesel from edible oils could disrupt the global balance between food supply and demand. Therefore, the use of non-edible oils as a feedstock for biodiesel production can help preserve edible oils for other food related industries. Biodiesel has gained international attention due to its renewability, biodegradability, carbon neutrality and non-toxic nature [2-5]. Developed countries like Malayasia, Brazil, Indonesia, USA, UK, Germany, Canada, have already adopted biodiesel blended with petroleum diesel. India also requires biodiesel as a self -reliant alternative to petroleum diesel [6-8].

In this context, the identification of fatty acid constituents in glycerides becomes crucial. Biodiesel primarily comprises methyl esters of long-chain fatty acids and is produced from non-toxic biological sources, such as vegetable oils and animal fats, through transesterification with methanol in the presence of a catalyst (which can be acid, base or enzyme such as lipase) [1,2,9,10]. Biodiesel offers several advantages and has the potential to address global warming and energy related challenges [1, 11-13]. Therefore, non-edible vegetable oils can serve as alternative feedstock for biodiesel production [14-17].

Sesamum indicum, known locally as Thoidong in Manipuri, is as annual flowering herb belonging to Pedaliaceae family (Figure 1 a). It is widely cultivated for its edible seeds, which is a major summer crop. This drought tolerant plant can grow in worst conditions where other crops may fail. Sesamum indicum seeds are among the among the richest oil content and have been a source of food and oil for thousands of years (Figure 1 b). The oil is highly stable with a long life due to its natural antioxidants. It is used in cooking, as salad oil, and in margarine, containing approximately 47% oleic acid and 39% linoleic acid. Sesamum indicium seeds are widely used in various cuisines and are added to bread and other dishes. In some regions, they can used to prepare dishes like Wangila and snakes

made with jaggery or sugar. In Manipur, black Sesamum indicum is used in traditional dishes like Thoidong and Singju. These seeds are also rich in various essential minerals, including copper, magnesium, manganese, calcium, phosphorus, zinc, iron, molybdenum and selenium.

Sesamum indicum seed oil has various applications, including massages for the skin and hair. It is used in the manufacture of Ayurvedic medicines. This aromatic oil is extracted from sesame seeds and contains linoleic and linolenic acids, as well as biologically active compounds like lignans, natural vitamin E and phytosterols. Industrially, Sesamum indicum oil can be used as a solvent in pharmaceuticals, aa carrier oil in cosmetics [19]. The oil also works synergistically with certain insecticides [20]. Lower-grade oil is used locally in soap, paint, lubricant and lighting applications. Sesamum indicum oil is a source of vitamin E and B6 [21].

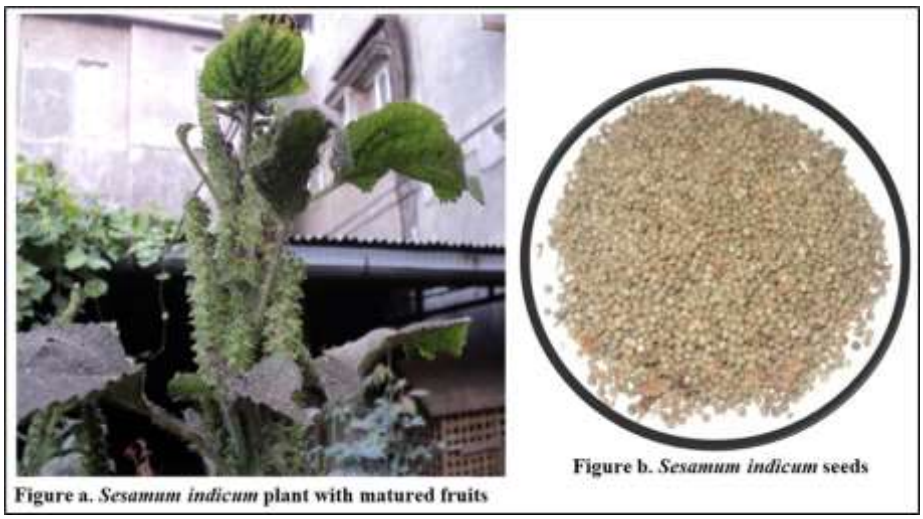

Figure 1: Sesamum indicum plant and seeds

2. Materials And Methods

Sesamum indicum seeds were collected from the region of Irong in Thoubal district, Manipur, India, situated at coordinates 24°37'48.00" N latitude and 94°01'12.00" E longitude during the harvest season, which spans from November to April. Initially, the seeds were subjected to cleaning and sun-drying for a period of 5 to 6 days. Subsequently, they were deshelled, and the kernel was crushed using a grinder prior to the extraction of oil. The methanol used in this process was of analytical grade, sourced from Mark in Mumbai, India. All other solvents and chemicals, also of analytical grade, were procured from commercial suppliers and were used as-is without further treatment.

The oil extraction was carried out from the crushed and powdered Sesamum indicum seed kernels using petroleum ether with a boiling point range of 40-60°C at a ratio of 10 ml per gram of material. This extraction process involved magnetic stirring at room temperature (approximately 29°C) and lasted for 4 hours and 30 minutes. The solvent was removed using a rotary vacuum evaporator (BUCHI Rotor vapour R-200) at 45°C to yield crude oil. This extraction process was repeated 2-3 times on the seed cake using fresh solvent for each iteration to maximize oil extraction. The extracted oil was further dried using a vacuum pump. Prior to transesterification, the oil was subjected to purification through column chromatography over silica gel with a particle size range of 60-120 mesh. The eluent used for this purification was a mixture of petroleum ether and ethyl acetate in a 20:1 ratio.

The property of glycerides were experimentally determined based on parameters such as density, colour, refractive index, acid value, iodine value, and saponification value, following the protocols outlined by the Association of Official Analytical Chemical Procedures [22]. Refractive indices of purified seed oils were determined by using the Abbe Refractometer (AW-24) at room of temperature, only two or three drops of oil was required. Densities of the purified oils were determined at room temperature (32°C). For this, a clean and empty plastic centrifuge tube was taken and weighed. Accurately 1000 μL (= 1 mL) of the liquid sample was transferred into the tube with the help of a syringe and then weighed again. Then the density is determined based on mass per unit volume of oil.

% oil content= (Weight of oil / Weight of powdered seeds) *100…… (1)

Acid value (mg KOH / g) = (56.1* V* N)/W …… (2)

where, V = titre value (mL)

N = normality of KOH solution (determined by standardizing KOH solution with oxalic acid).

W = weight of test sample taken in g.

Iodine value = (12.69 * N* (V_B-V_S))/W…… (3)

Where, VB = Volume of sodium thiosulphate solution used for the blank (mL)

VS = Volume of sodium thiosulphate solution used for the oil sample (mL),

N = Normality of sodium thiosulphate solution used,

W = Weight of oil sample taken in g

Saponification value = (56.1* M* (V_B-V_S))/W…… (4)

where, VB = Volume of 0.5 M HCl solution used for the blank (mL)

VS = Volume of 0.5 M HCl solution used for the oil sample (mL)

M = Molarity of HCl used

W = Weight of oil sample taken in g

% Moisture = (W_1 - 〖 W〗_(2))/W_1 * 100…… (5)

where, W1 = Initial weight of oil,

W2 = Final weight of oil

Study of Physical Parameters of Sesamum Indicum Seed Oil

The refined oil underwent a process of transesterification to convert it into fatty acid methyl esters (FAME) using a catalyst known as Athia, derived from banana plants specifically, ashes from the Musa balbisiana banana fruits, comprising 20% of the oil [18]. The oil was mixed with methanol (at a ratio 10 mL per 1 gram of oil) and the catalyst (constituting 20% of the oil). The mixture was stirred vigorously using magnetic agitation at room temperature (260C) and the completion of the reaction was monitored through thin layer chromatography (TLC).

Table 1: parameters of *Sesamum indicum* seed oil calculated using equation (1-5)

Physical Parameters	Observed Values
Colour	light yellow
Oil content (wt. %)	43.53
Density (g/cm^3)	1.0425
Acid Value (mg KOH/g)	1.601
Iodine value (gI_2/100g)	80.32
Saponification value (mg KOH/g)	190.81
Refractive Index	1.4632
Moisture (%)	0.109

Upon concluding the reaction, the resulting mixture was subjected to extraction using petroleum ether (with a boiling point 40-60^{0}C). The organic layer was rinsed with brine, dried over anhydrous sodium sulphate overnight and the solvent was removed under vacuum to yield the raw product. The crude product was subsequently subjected to further purification through column chromatography using silica gel, employing a mixture of petroleum ether and ethyl acetate (inn a 20:1 ratio) as the eluent. The product was concentrated and evaporated to dryness using a rotary evaporator and any remaining solvent traces were removed through a vacuum pump, resulting in pure biodiesel (FAME). The composition off the FAME mixture was determined using Perkin Elmer Clarus 600 GCMS. The column utilized was Elite 5MS, initially held at 14^{0}0C for a certain period, then raised to 240^{0}C at a rate of 4^{0}C/min, and held for additional 5 minutes. The injector and source temperature were maintained at 250^{0}C and 150^{0}C respectively. Helium was used as a carrier gas with a total scan time of 35 minutes. The electron impact (EI) mode of ionization was applied and the mass range covered was from 20 to 400 Da. For the identification of FAME, a library search was conducted utilizing resources such as National Institute of Standards and Technology (NIST), National Bureau of Standards (NBS) and the Wiley GCMS library. The Fatty acid profile of biodiesel obtained Sesamum indicum seed oil is provided in Table 2. The 1H and 13C NMR spectra was acquired using Carbon Deuterium Trichloride ($CDCl_3$) at 300 MHz with a 5 mm NMR spectrometer, and the IR spectrum was recorded as a thin film on a KBr plate using a Perkin Elmer RXIFT-IR spectrometer.

The fatty acid composition of the FAME derived from Sesamum indicum seed oil was analysed through GC-MS. Each peak observed in the gas chromatogram was scrutinized and the respective fatty acid was identified using the MS database. Each peak corresponds to a distinct fatty acid methyl ester. The gas chromatogram displayed three distinct peaks, signifying the presence off three different fatty acids methyl esters. The peak located to the right in the mass spectrum of any fatty acid methyl ester provided the molecular weight of the fatty acid and is referred to as the molecular ion peak. The retention time is the duration taken for the development of each peak and the base peak designates the peak with the highest relative abundance inn the mass spectrum. Generally, the peak with highest m/z value is indicative of the molecular ion peak.

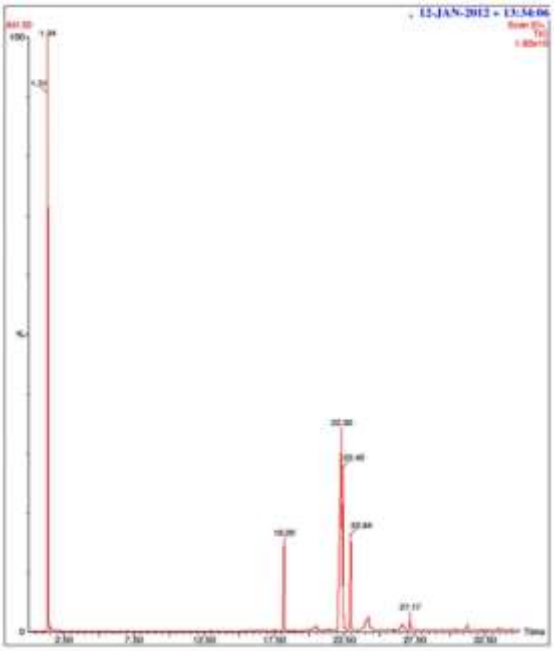

Figure 2: Gas chromatogram of biodiesel from *Sesamum indicum* seed oil

3. Results and Discussion

The yield of the extracted and purified glycerides from *Sesamum indicum* seed oil was found to be 43.53wt % at the room temperature (29°C) within 4:30 hours while the yield of trans esterified glyceride known as Fatty Acid Methyl Ester (FAME) was 81.2 wt.% at the room temperature (31°C) within 2:15 hours. The light-yellow colour of the *Sesamum indicum* seed oil was due to the presence of natural pigments like tocopherols, carotenoids and their derivatives. The yield of the oil was moderate. Density and iodine value of *Sesamum indicum* seed oil were found to be 1.0425 g/cm^3 and 80.32 gI_2/100 respectively which are comparable to those of soya bean oil and sunflower oil. The acid value of this oil was found to be 1.601 mg KOH/g which is within the limit for industrially useful oil. Saponification value was 190.81 mg KOH/g whose value is suitable for soap making and cosmetic industries. Refractive Index of this oil was 1.4632 which is not very much different from those recorded for conventional seed oils such as palm oils (1.445-1.451), cotton seed oil (1.468-1.472), safflower oil (1.473-1.476) and soya bean oil (1.4728) at 25'C. Moisture was found to be 0.109% (low value) which is suitable good quality and contamination does not take place easily due to its low value of moisture. Low moisture content is an essential criterion for commercial oil.

Analysis of FAME of *Sesamum indicum* Seed oil

^{1}HNMR (300 MHz, CD Cl_3): δ 5.26 -5.39 ppm, δ 7.26 ppm, δ 2.78 ppm, δ 2.33 ppm, δ 2.01 -2.09 ppm, δ 1.25 – 1.30 ppm, δ 1.25 -1.30 ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ 173.12 ppm, δ 126.94 ppm, δ 127.58 ppm, δ 127.72 ppm, δ 128.07 ppm, δ129.84 ppm, δ130.06ppm, δ 131.79 ppm, 61.94 ppm and 13.96 -34.02 ppm. FT-IR (thin film): 1724 cm^{-1}, 1195 cm^{-1}, 1612 cm^{-1}, 2748 cm^{-1}, 2925 cm^{-1}, 3057 cm^{-1}, 704 cm^{-1}. Relative percentages of fatty acid esters were calculated from the total ion chromatography by computerized integrator and results are presented (Table 2). Fatty Acid Methyl Ester (FAME) from Sesamum indicum consists of 15.35 wt.% of methyl palmitate (C16:0), 67.03 wt.% of methyl linoleate (C18:2), 14.92 wt.% of methyl oleate (C18:1) & 2.70 wt.% of methyl stearate (C20:0).

Table 2: Composition of biodiesel from *Sesamum indicum* seed oil

Retention time (mm)	FAME	wt.%
22.18	Methyl palmitate	15.35
22.21	Methyl linoleate	67.03
22.89	Methyl stearate	14.92
27.15	Methyl arachidate	2.70

The mass spectra of methyl palmitate, methyl linoleate, methyl stearate and methyl arachidate are shown in Figure 3a to 3d. The molecular ion peaks and base peaks are presented (Table 3).

Table 3: Molecular ion and base peaks of FAME from *Sesamum indicum* seed oil

Fame	Molecular Ion Peak (M/Z)	Base Peak (M/Z)
Methyl palmitate	270	74
Methyl linoleate	294	67
Methyl stearate	298	74
Methyl arachidate	326	74

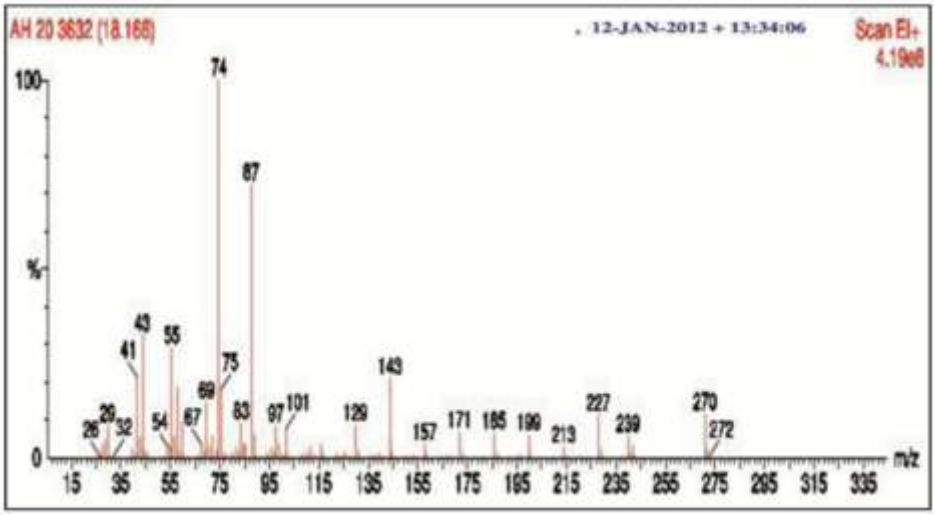

Figure 3 (a): Mass spectrum of methyl palmitate

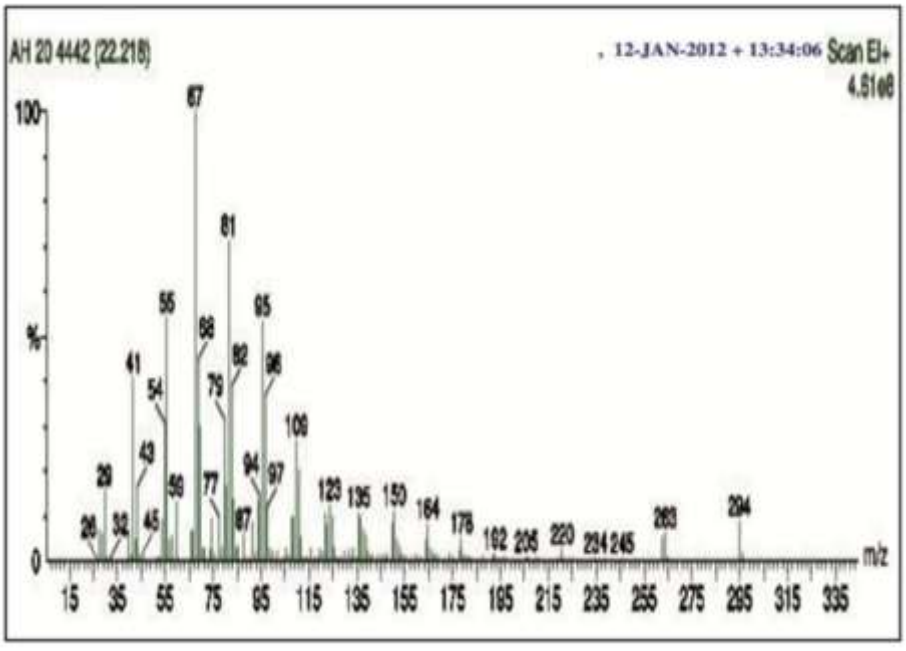

Figure 3 (b): Mass spectrum of methyl linoleate

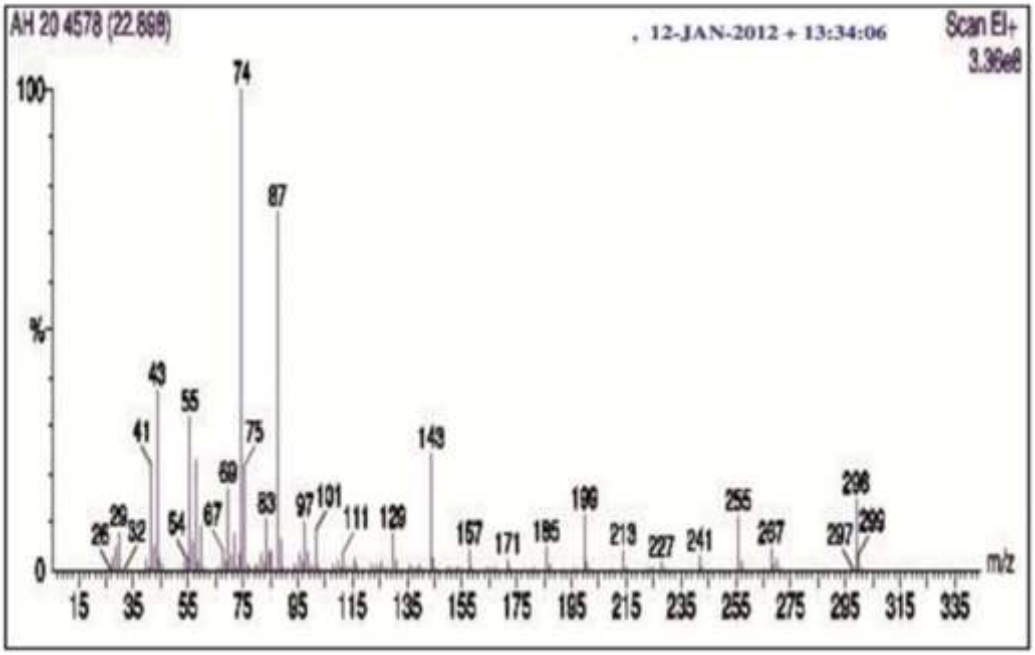

Figure 3 (c): Mass spectrum of methyl stearate

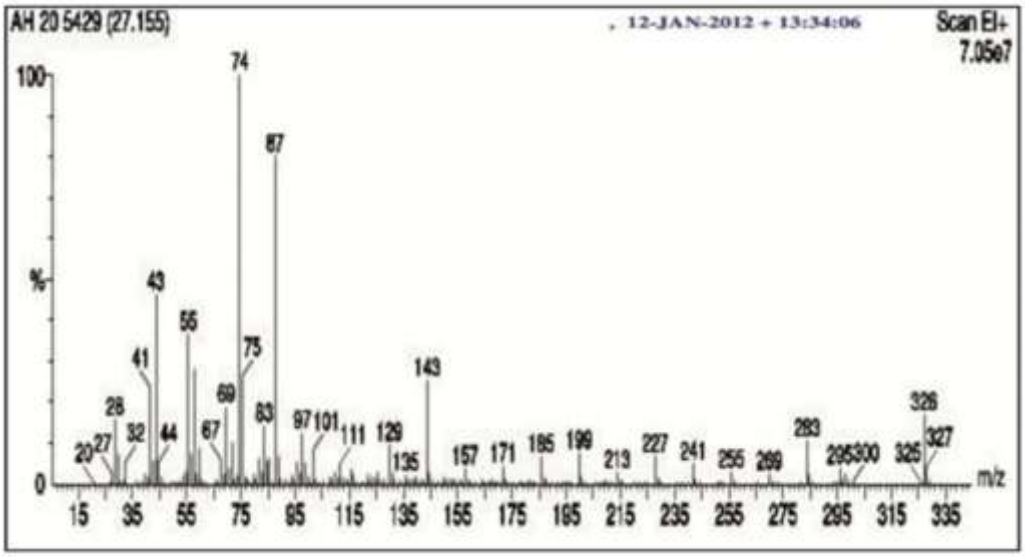

Figure3 (d): Mass spectrum of methyl arachidate

The ^{1}H NMR spectrum of biodiesel from *Sesamum indicum* seed oil is shown in the Fig.4. The multiplet at δ 5.26 - 5.39 ppm suggests the olefinic protons (–CH=CH–). A singlet at δ 7.26 ppm indicates methoxy protons of the ester functionality of the biodiesel. The triplet at δ 2.78 ppm (t, ^{3}J=6.0 CH_2) suggests the bis-allylic protons (–C=C–CH_2=C=C–) of the unsaturated fatty acid chain. The triplet at δ 2.33 ppm (t, ^{3}J=75 CH_3) indicates the α-methylene protons to ester (–CH_2–CO_2CH_3). The α- methylene protons to double bond (–CH_2–C=C–) is seen as a multiplet at δ 2.01 – 2.09 ppm. The β-methylene protons to ester (CH_2–C–CO_2CH_3) also appear at as a multiplet at δ 1.25 - 1.30 ppm. The singlet signals at δ 1.25 - 1.30 ppm are due to the protons of backbone methylene of the long fatty acid chain. The terminal methyl protons (C–CH_3) at 0.85 - 0.90 ppm appear as a multiplet. Figure 5 shows the ^{13}C NMR spectrum of biodiesel from *Sesamum indicum* seed oil. The signal at δ 173.12 ppm suggests the carbonyl carbon of the ester molecular and the olefinic carbons appear at δ 126.94, 127.58, 127.72, 128.07, 129.84, 130.06 and 131.79 ppm. The signal at δ 61.94 ppm is due to methoxy carbons of esters. The methylene and methyl carbons of fatty acids moiety appear in the range from δ 13.96 - 34.02 ppm. In Figure 6, the IR spectrum of biodiesel from *Sesamum indicum* seed oil is shown. IR spectrum of biodiesel shows a C=O stretching frequency of methyl esters at 1724 cm^{-1} and C–O stretching band at 1195 cm^{-1}. The weak signal at 1612 cm^{-1} may be due to C=C

stretching frequency. Strong and sharp signals at 2748 and 2925 cm^{-1} are due to C–H stretching frequencies. The absorbance at 3057 cm^{-1} suggests the =C–H stretching frequency. The observation of an absorption peak at 704 cm^{-1} indicates the CH_2 rocking.

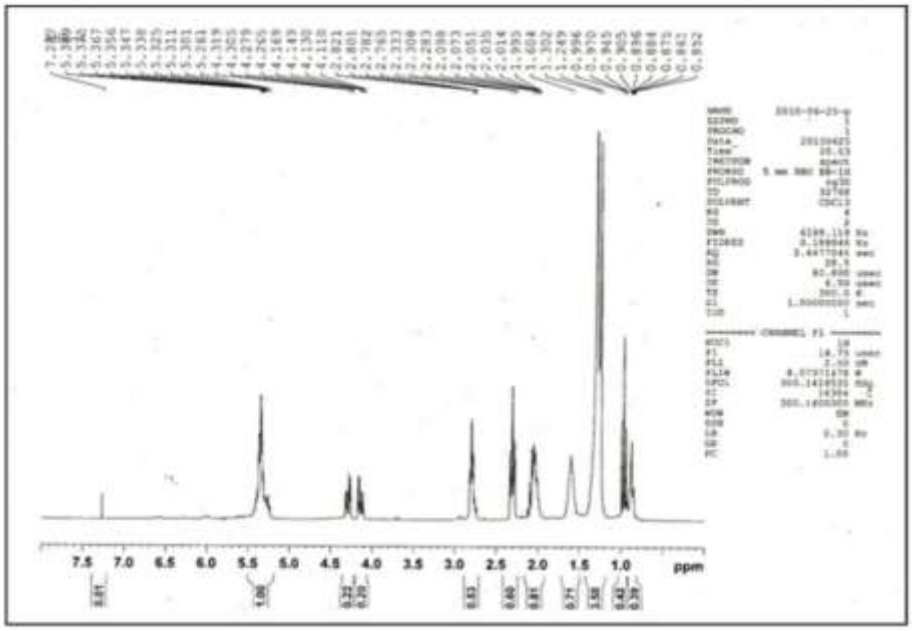

Figure 4: ^{1}H NMR spectrum of biodiesel from *Sesamum indicum* seed oil

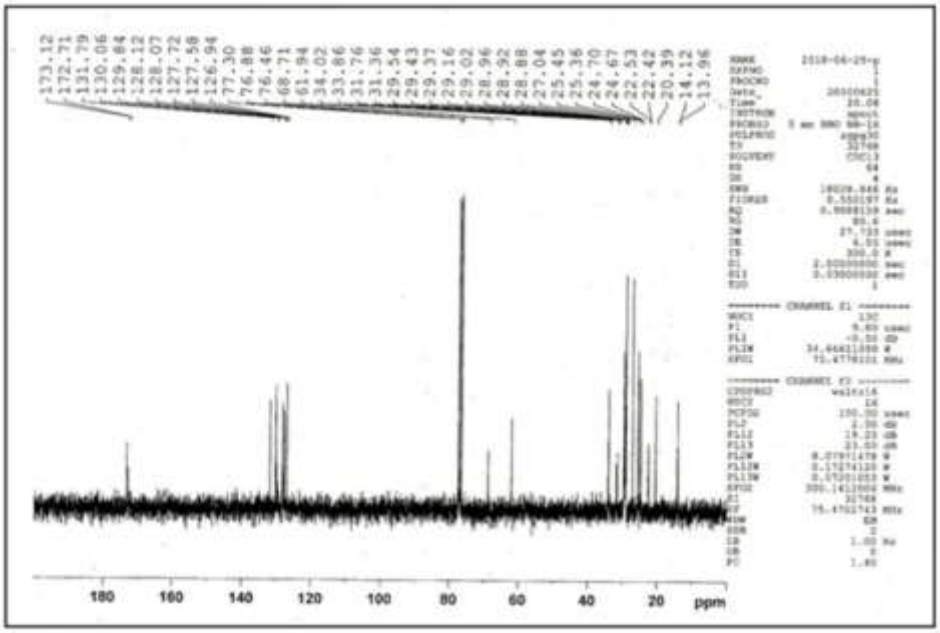

Figure 5: ^{13}C NMR spectrum of biodiesel from *Sesamum indicum* seed oil

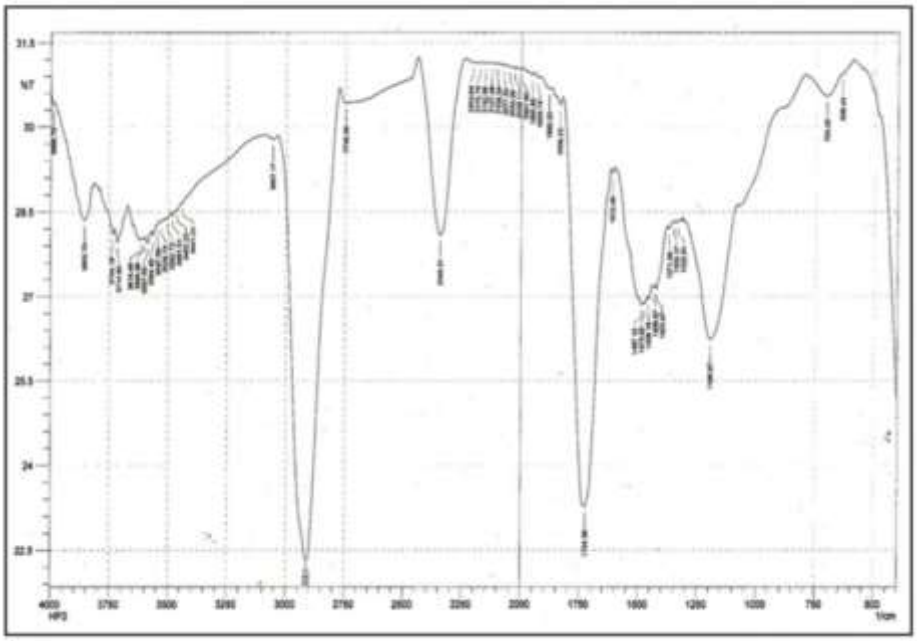

Figure 6: IR spectrum of biodiesel from *Sesamum indicum* seed oil

Theoretical determination of IV, SN and CI of FAMEs

Three important physical properties of biodiesel, viz. iodine value (IV), saponification number (SN) and cetane index (CI) were performed applying theoretical calculation based upon fatty acid profile shown in the Table IV. The IV, SN and CI of FAMEs were calculated using equations (6), (7) and (8) respectively. Results are shown in Table 4.

$$IV = \sum(254 \times D \times Ai)/MWi \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$SN = \sum(560 \times Ai)/MWi \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$CI = 46.3 + \frac{5458}{S} - 0.225R \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Where, D = number of double bonds in the ith component

Ai = percentange of the ith component in the chromatogram

MWi = molecular weight of the ith component of the FAME in the oil

S = saponification number (SN) as calculated by the equation (7)

R = iodine value (IV) as calculated by equation (6)

Table 4: Experimentally and theoretically as calculated IV, SN, CI of FAME Profile of *Sesamum indicum* plant

Name of the oil plant	IV (g/100g)	SN (mg KOH / g)	CI
Sesamum Indicum	115.82	190.96	54.63

4. Conclusion

The yield of the extracted and purified glycerides from *Sesamum indicum* seed oil was found to be 43.53wt % at the room temperature (29°C) within 4:30 hours while the yield of trans esterified glyceride known as Fatty Acid Methyl Ester (FAME) was 81.02 wt. % at the room temperature (31°C) within 2:15 hours. The colour, density, acid value, iodine value, saponification number, refractive index and moisture of the *Sesamum indicum* seed oil were found to be light yellow, 1.0425 g/cm^3, 1.601 mg KOH/g, 80.32 gI_2/100 g, 190.81 mg KOH/g, 1.4632 and 0.109 % respectively. The biodiesel from *Sesamum indicum* seed oil, after extraction and purification by column chromatography, was prepared by heterogeneous transesterification process and analysed for its fatty acid methyl esters composition using IR, NMR and GC-MS. It was also found that FAME from *Sesamum indicum* seed oil consists of 15.35 wt. % of methyl palmitate (C16:0), 67.03 wt. % of methyl linoleate (C18:2), 14.92 wt.% of methyl stearate (C20:0) and 2.70 wt. % of methyl arachidate

(C20:0). The iodine value, saponification number, cetane index of the biodiesel from oil seeds of *Sesamum indicum* were 115.82 (g/100g), 190.96 (mg KOH/g) and 54.63 respectively. The molecular ion peak of methyl palmitate, methyl linoleate, methyl stearate and methyl arachidate, were observed at 270, 294, 298, and 326 respectively as was expected.

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